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(54) Title: A PROKARYOTIC PROTEIN HAVING FUNCTIONAL AND STRUCTURAL HOMOLOGY WITH THE HUMAN P-GLYCOPROTEIN ENCODED BY THE MDR-1 GENE, NUCLEIC ACIDS ENCODING AND CELLS EXPRESSING SAID PROTEIN

(57) Abstract

The present invention relates to protein and genes encoding said protein as well as cells harbouring said genes or protein. The protein is a prokaryotic homolog of the human P-glycoprotein which is encoded by the MDR-1 gene. The MDR-1 gene is a gene related to the occurrence of multidrug resistance.

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Title: A prokaryotic protein having functional and structural homology with the human P-glycoprotein encoded by the MDR-1 gene, nucleic acids encoding and cells expressing said protein.

The present invention relates to a novel protein and genes encoding said protein as well as cells harbouring said genes or protein. The protein is a prokaryotic homolog of the human P-glycoprotein which is encoded by the MDR-1 gene. The MDR-1 gene is a gene related to the occurrence of multidrug resistance.

Multidrug resistance poses a serious clinical problem in the treatment of cancer and infectious diseases and is responsible for many tens of thousands of deaths each year (*1*). Resistance of human cancer cells is commonly associated with high expression levels of the MDR1-encoded P-glycoprotein (2). Indeed, transfection experiments demonstrate that overexpression of P-glycoprotein alone can confer multidrug resistance to an otherwise drug-sensitive cell line. P-glycoprotein is a member of the ATP-binding cassette (ABC) superfamily of transporters, whose functions include the ATP-dependent extrusion of amphiphilic compounds out of the cell (3).

Much work has been devoted over the years to the identification of the protein and gene encoding it, responsible for multidrug resistance. After the identification the questions have shifted to identification of the mechanisms by which the now identified P-glycoprotein works, what determines the binding of drugs to be transported, what determines the specificity and how such mechanisms can be influenced. The question also remains how multidrug resistance can be avoided or which drugs may be able to avoid being pumped out of the cell through the P-glycoprotein pathway. All these questions have been found very difficult to tackle, because unfortunately functional (over)expression has been very difficult to achieve owing to toxic effects of

P-glycoprotein expression in E.coli (4), thereby seriously hampering the creation of a model system to elucidate the questions previously mentioned.

The appeal of using a prokaryote such as E.coli to investigate the molecular mechanism of P-glycoprotein is twofold: First, the overexpression and purification of large amounts of the protein can be achieved more rapidly than if carried out in eukaryotic cells. Second, the expression of P-glycoprotein in E.coli will allow the rapid identification by selection or by molecular genetics of mutant P-glycoproteins with altered drug specificity or transport properties. The present invention now provides a model system for studying the mechanisms based on a novel prokaryotic homolog of the human P-glycoprotein and the gene encoding it.

Prokaryotes, in particular bacteria have developed fascinating mechanisms to resist antibiotics and other cytotoxic drugs (1). In previous work, we discovered that one mechanism of drug resistance in *Lacococcus lactis* may be similar to multidrug resistance in mammalian cells, namely the mediation of drug efflux by an ATP-dependent efflux pump (5, 6). This notion prompted us to search for a (putative) P-glycoprotein-like gene in *L.lactis*. The lactococcal P-glycoprotein homolog was discovered in tne course of work on the *apl* gene of *L.lactis* MG1363, which encodes an alkaline phosphatase-like enzyme. Analysis of a chromosomal DNA fragment containing *apl* revealed a convergently transcribed, 3' adjacent open reading frame of 1770 bp, designated *lmrA*. The *lmrA* gene encodes a polypeptide of 590 amino acids with a calculated molecular mass of 64,613 Dalton (Fig. 1). Hydropathy analysis of LmrA suggests the presence of an N-terminal hydrophobic domain with 6 (putative) transmembrane regions and a C-terminal hydrophilic domain (data not shown). This latter domain contains diagnostic features of an ABC-type ATPase, such as the ABC signature sequence, and the Walker A and B motifs (7).

Comparison of LmrA and various ABC transporters clearly identifies the lactococcal protein as a homolog of the human

P-glycoprotein (Fig. 1). This 1280-residue membrane protein is predicted to contain two homologous halves, each with 6 transmembrane regions and a large cytoplasmic loop containing an ABC ATPase (8). Amino acid alignment of LmrA and each half of P-glycoprotein indicates that they share 32% identity with an additional 16% conservative substitutions (9) for an overall similarity of 48%. Interestingly, the sequence identity includes particular regions in the hydrophobic domains (e.g, the region comprising transmembrane segment V and VI) which, in P-glycoprotein, have been implicated as determinants of drug recognition and binding (10). LmrA is to a lesser extent similar to other ABC transporters. In this case, however, the sequence identity is almost exclusively confined to the hydrophilic ABC domains.

LmrA thus defines a new class of multidrug resistance proteins in prokaryotes and offers amongst many other things, an exciting model system to study the molecular mechanism of P-glycoprotein-mediated drug transport.

Thus, the present invention provides a recombinant or isolated nucleic acid molecule derived from a prokaryotic gene, said recombinant nucleic acid molecule encoding at least a specific and/or functional part and/or derivative of a transporter protein, which transporter protein has functional and/or structural similarity with the P-glycoprotein encoded by the human MDR1 gene. As stated before, this molecule can be used to produce a model system to study the mechanism by which it human equivalent works. However, this is not the only purpose for which this molecule or its products can be used. Many uses of the gene, or parts thereof, the products (or parts of such products), cells provided with such genes and/or products will be readily apparent to the person skilled in the art.

Below a number of possible applications is given which is by no means intended to be exhaustive. Generally the uses will be based on the function, the structure or other properties of the molecules according to the invention, especially in relation to multidrug resistance, ATP dependent pumping of

(amphiphilic) substances, localization of in particular the polypeptide over membranes, etc. especially in relation to the human homolog functions. Apart from the function of the products according to the invention, which can be readily used by the skilled artisan to identify whether a products falls within the scope of the present invention, there is a separate, not necessarily related criterion for the molecules according to the invention, which criterion comprises the structural characteristics of the molecules.

Generally speaking for nucleic acid molecules, i.e DNA and/or RNA the structural homology between molecules according to the invention when compared with at least a specific part of the sequence of fig.5 should be high enough to allow for hybridization between such a molecule and a nucleic acid molecule having at least such a part of fig.5 or the complementary strand thereof under relatively stringent conditions. Generally speaking this is the case for a homology of over 70%, preferably 80% or even 90%. If the homology in a part of such a molecule is above the levels just stated, it may be lower in other areas of such a molecule. Preferred molecules according to the invention in some aspects, are those wherein the structural similarity with the human P-glycoprotein is at least 40% overall, preferably those wherein the structural similarity with the human P-glycoprotein comprises at least 30% identity overall.

Another, possibly overlapping group of preferred molecules according to the invention is the group which encodes ATP-dependent efflux pump activity when expressed in a suitable host cell.

The groups of molecules according to the invention mentioned above are very suitable for transfer into a host cell whereby they can be used to study the mechanism of ATP-dependent pumps, to identify cytotoxic agents not susceptible to extrusion by such a pump, but also to provide host cells with multidrug resistance. Derivatives of the nucleic acid molecules can be made which encode pumps with different specificity or activity, antagonists for the pumps

can be produced,e.g. by blocking the pumps with antibodies, etc.

As may be apparent from the above it is preferred that the nucleic acid molecule according to the invention, encodes a transmembrane protein.

It will however be clear to the skilled artisan that it is also possible to use nucleic acids which do not encode a protein, or which are not used to produce proteins. These nucleic acids are used to detect or amplify other nucleic acids which encode the same or similar polypeptides as those according to the invention. These detection and/or amplification methods, such as hybridizations and PCR are well known in the Art.

For the polypeptides according to the invention to be functional as an ATP dependent pump, it is probably required that they be positioned in a suitable manner in a membrane. For such positioning signal sequences for transport of the protein to its position may be required. Therefore such signal sequences are preferably present on the protein, be it the natural signal sequences or others which have been somehow attached to a part of the proteins according to the invention. These altered proteins are within the scope of the present invention being derivatives. Other derivatives include amino acid changes, be it conventional and having no significant effect on structure and/or function of the protein, or be it changes which do influence binding capacity, specificity, pumping activity or structure of the proteins.

Very suitable for use in a model system according to the invention is a nucleic acid molecule as disclosed above originating from a lactic acid bacterium, especially originating from the genus *Lactococcus*, more in particular originating from *Lactococcus lactis*.

The latter nucleic acid molecule preferably comprises at least a functional and/or specific part of the following sequence.

Polypeptides according to the invention may be obtained in any suitable manner, be it by isolation, chemical synthesis or

transcription and/or translation of a nucleic acid molecule according to the invention.

Transcription and/or translation can be suitably done in a host cell, although in vitro systems are also available. For expression in a host cell suitable vehicles comprise recombinant vectors comprising a nucleic acid molecule according to the invention and suitable regulatory elements for expression of the nucleic acid molecule and/or elements for transfer into host cells and/or replication in host cells.

These elements, such as promoters, terminators, enhancers, repressors and the like are all well known in the art. They should of course be operable in the host cell in which the expression should take place. They may be autologous to the host cell, or to the nucleic acid according to the invention, or completely heterologous. For the model system it is preferred that the host cell is autologous to the nucleic acid and that the regulatory elements are also from the same organism. This may also be preferred in other uses. However, if a cell is to be provided with (additional) multi drug resistance activity, some elements, including the nucleic acid encoding said activity will be (preferably) heterologous. Especially in this case it will be preferred that the expression of the multi drug resistance be inducible, preferably capable of being essentially completely switched on or completely switched off.

Another aspect of the invention are recombinant host cell comprising a nucleic acid molecule and/or a vector according to the invention. These can be the cells of the model system, or cells provided with (additional) multidrug resistance.

A preferred embodiment of the invention is a recombinant host cell comprising a transmembrane prokaryotic polypeptide having functional and/or structural similarity with the human P-glycoprotein encoded by the MDR-1 gene.

The cells according to the invention can also be used to produce a functional polypeptide having functional or structural similarity with the human P-glycoprotein encoded by the MDR1 gene, by culturing such a cell in a suitable culture

medium and harvesting the polypeptide from the culture. The polypeptides may also be harvested when residing in a membrane, in microsomes or other vesicles, in which they are most likely to be active.

Polypeptides having functional or structural similarity with the human P-glycoprotein encoded by the MDR1 gene obtainable by such a method are also part of the present invention, as are recombinant or synthetic polypeptides comprising at least so many amino acid residues as to be functional as or specific for a polypeptide having functional or structural similarity with the human P-glycoprotein encoded by the MDR1 gene.

The invention further comprises an antibody or a specific binding part and/or derivative thereof recognizing an epitope associated with a polypeptide according to the invention.

The invention also comprises the use of a polypeptide or a cell according to the invention in determining whether a substance can inhibit or escape multi drug resistance, as well as methods for determining whether a substance is capable of inhibiting transport of cytotoxic substances from a cell, comprising contacting said substance with a polypeptide or a cell according to the invention.

The invention further provides methods for providing cells with (additional) multidrug resistance, comprising providing the cells with a transmembrane polypeptide as disclosed hereinabove. Preferably the transmembrane polypepitde is provided by expression in the cell of a nucleic acid as disclosed herein.

EXPERIMENTAL.

MATERIALS AND METHODS

The isolation of the *L.lactis* MG1363 genomic DNA clone (6.0 kb *Sau*3A DNA fragment in the *E.coli* cloning plasmid pUC19) containing the *apl* and *lmrA* genes will be described elsewhere. Nucleotide sequence analysis was performed on double stranded DNA using the dideoxynucleotide chain-termination procedure (21). PCGENE (release 6.8, Genofit) was used for computer-assisted analysis of nucleotide and protein sequences. Amino acid substitutions said to be conserved are: A, S, T; D, E; N, Q; R, K; I, L, M, V; F, Y, W. Protein secondary structure was predicted using the algorithm of Kyte and Doolittle (22).

Plasmid pGKLmrA was constructed by subcloning the 2.3 kb *Sph*I-*Pvu*II fragment, containing *Imr*A, into the plasmid pGK13 harboring a chloramphenicol resistance marker for positive selection (23). *E.coli* CS1562 (*tol*C6::Tn10)(26) was transformed with plasmid DNA by electroporation (21). Transformants were selected on Luria broth supplemented with 25 mM glucose, 16 µg/ml tetracycline, and 9 µg/ml chloramphenicol. The sensitivity of transformants to various drugs was assessed by inoculating exponentially growing cultures (1:100) into 96-well plates containing serial dilutions of the drugs in the liquid medium described above. The growth rate at a given drug concentration relative to growth in its absence was determined as a function of the drug concentration. For Northern blot analysis, total RNA of transformants was isolated as described (21), 30 µg of which was fractionated on a 2.2 M formaldehyde-1.2% (w/v) agarose gel, transferred to Qiabrane membrane (Qiagen, Westburg, NL) and hydridized to the 2.3 kb *Sph*I-*Pvu*II DNA fragment. Transcript sizes (kb) were estimated using a 0.24 - 9.5 kb RNA ladder (Gibco BRL, Paisley, UK).

To study LmrR-mediated drug transport, cells were harvested in the mid-exponential phase, washed and resuspended

in 50 mM potassium-HEPES (pH 7.5) supplemented with 3 mM $MgSO_4$. Inside-out membrane vesicles were prepared as described (24) and resuspended in the HEPES buffer described above. Ethidium transport in cells and membrane vesicles was measured using fluorescence spectrometry (25). The uptake of [^{3}H(G)]daunomycin (96.2 GBq/mmol, New England Nuclear, NL) and *N*-(4',4'-azo-*n*-pentyl)-21-deoxy (APD)-[21-^{3}H]ajmalinium (46 GBq/mmol) in membrane vesicles was assayed via the filtration method. The membrance potential ($\Delta\psi$) in cells was measured using a tetraphenylphosphonium (TTP^+)-selective electrode (26) in the presence of 50 µM reserpine to inhibit the LmrA-mediated extrusion of TPP^+. All experiments were performed at least in triplicate. Standard deviations were calculated where possible, and these are indicated as ± SD or as error bars in the figures. Having established the structural similarity between LmrA and P-glycoprotein, we began to explore the function of LmrA. For this purpose, *lmrA* was subcloned into the E. coli/L. lactis shuttle vector pGK13 (11), giving pGKLmrA. Control and *lmrA*-containing plasmids were transferred to the drug-sensitive *E.coli* strain CS1562, the increased outer membrane permeability of which is due to a deficiency in the TolC porin (12). Northern blot analysis was performed to confirm the expression of *lmrA* in this host. Using an *lmrA* gene-specific DNA probe, the 1.8 kb *lmrA* messenger was readily detectable in cells harboring pGKLmrA (Fig. 2). The signal was absent in the parental vector control.

Two approaches were used to assess the ability of heterologously expressed LmrA to act as a multidrug extrusion system: (i) in vivo resistance to growth inhibition by lipophilic cations, and (ii) transport of lipophilic cations. *E.coli* CS1562/pGK13 is unable to grow on solid media containing ethidium at concentrations above 20 µM. Strikingly, cells harboring pGKLmrA are able to form colonies on plates containing 60 µM ethidium after overnight incubation at 37°C. This difference in in vivo drug resistance was studied more extensively in batch cultures in the presence of ethidium, daunomycin, rhodamine 6G, or tetraphenyl phosphonium (TPP^+)

which are known substrates of P-glycoprotein (13). The growth rate at a given drug concentration relative to growth in its absence was determined as a function of the drug concentration. The results, a typical example of which is depicted in Fig. 3A, show that the IC_{50} values (the drug concentrations required to inhibit the growth rate by 50%) for *E.coli* CS 1562pGKLmrA are at least 30-fold higher than those for the control cells harboring pGK13.

To elucidate the mechanism of LmrA-associated drug resistance, fluorimetric ethidium transport assays were performed (5). Washed cell suspensions of *E.coli* CS1562 containing pGKLmrA or pGK13 accumulated ethidium at the same initial rate (Fig. 3B). In the control cells, this membrane potential ($\Delta\psi$)-driven passive influx of the lipophilic cation was enhanced upon energization with glucose. due to the increase of the $\Delta\psi$(interior negative) from -67 to -90 mV (11). Although comparable changes of the $\Delta\psi$ were observed in LmrA-expressing cells, energization with glucose resulted in the extrusion of ethidium rather than uptake. Hence drug resistance in LmrA-expressing cells is based on active drug efflux.

The energetics and specificity of LmrA-mediated drug transport were studied in more detail in inside-out membrane vesicles. The uptake of daunomycin above equilibration levels was observed in membrane vesicles of LmrA-expressing cells in the presence of ATP, an ATP regenerating system, and the ionophores valinomycin plus nigericin which selectively dissipate the components of the proton motive force (Fig. 3C). Daunomycin was not accumulated in these membrane vesicles in the presence of ATPγS, a non-hydrolyzable ATP analog, indicating that ATP hydrolysis is required for transport. This conclusion was confirmed by the inhibition of active daunomycin uptake by *ortho*-vanadate, an inhibitor of ABC and P-type ATPases (Fig.3D). Membrane vesicles prepared from control cells did not display the ATP-dependent uptake of daunomycin (Fig. 3C). Similar results were obtained for the transport of N-(4',4'-azo-*n*-pentyl)-21-deoxy (APD)-ajmalinium,

a high-affinity substrate of P-glycoprotein (15), and ethidium (data not shown). The inhibition of daunomycin uptake in membrane vesicles of LmrA-expressing cells by a 12-fold excess of ethidium, rhodamine 6G, or TPP^+ points to competition between these substrates for transport by LmrA (Fig. 3D). Finally, LmrA-mediated drug transport was inhibited by reserpine, a well-known inhibitor of P-glycoprotein.

Our results identify LmrA as an ATP-dependent multidrug transporter which represents a naturally occurring, functional 'half-molecule' of P-glycoprotein. Resistance of *Lactococcus lactis* to cytotoxic compounds thus shares features with the multidrug resistance phenotype of mammalian tumor cells. Here, we report the gene cloning and functional characterization in Escherichia coli of LmrA, lactococcal homolog of the human multidrug resistance P-glycoprotein.

In subsequent studies, LmrA protein was expressed in the human lung fibroblast cell line GM00637 (data not shown). This cell line has a negligible expression level of P-glycoprotein. The *lmrA* gene was inserted into the pCI-neo mammalian expression vector under the control of the human cytomegalovirus immediate-early enhancer/promoter region. Confocal fluorescence microscopy revealed the presence of LmrA protein in the plasma membrane of these cells. Transient expression of LmrA conferred a 10 to 60-fold resistance on GM00637 cells to amphiphilic drugs such as ethidium, daunomycin, rhodamine 123, colchicine, and vinblastine. Vinblastine and Hoechst 33342 transport assays identified drug extrusion across the plasma membrane as the underlying mechanism of drug resistance in LmrA-expressing GM00637 cells. LmrA-mediated drug resistance in GM00637 cells could be reversed by modulators of P-glycoprotein, such as reserpine, verapamil, nicardipine, cyclosporinA and quinidine. Thus, the drug resistance phenotype of LmrA and MDR1-expressing cells is similar, if not identical. The functional properties of LmrA have been studied in more detail upon expression of the protein in insect cells (*Sfodoptera frugiperda*). Analysis of the kinetics of vinblastine binding to LmrA, and of the effect

of modulators on vinblastine binding indicate that LmrA contains multiple drug and modulator binding sites. These sites are similar to those in the human P-glycoprotein (data not shown).

Although true P-glycoprotein homologs have not yet been found in bacteria other than *L.lactis*, there is little doubt that many more remain to be identified. All such systems can play a role in drug resistance. A typical example is the development of chloroquine resistant phenotypes of the malaria parasite *Plasmodium falciparum* which has been linked to the overexpression of two P-glycoprotein homologs (16). Equally worrisome is this development in bacteria, which can easily exchange genetic information. If one bacterium can develop resistance by overexpression of a P-glycoprotein homolog, others (including pathogenic ones) are likely not far behind.

Appreciation of the mechanisms through which eukaryotic and prokaryotic cells develop drug resistance is critical for the development of effective new drugs. LmrA offers an exciting model system that can provide an important framework for interpreting data obtained on its medically important counterparts in humans and pathogenic microorganisms. In addition, the lactococcal P-glycoprotein homolog will enable the rapid screening and identification of compounds that have potential as modulators of the *in vivo* transport activity of P-glycoprotein and related systems. The rational design of such compounds poses a key challenge for the future.

LEGENDS TO THE FIGURES

Fig. 1. Comparison of the amino acid sequence of LmrA and the N- and C-terminal half of the human multidrug resistance P-glycoprotein MDR1 (3). MDR1-N and MDR1-C represent amino acid residues 1 to 640 and 641 to 1280 of MDR1, respectively. The last residue in each row is numbered. A dark background indicates identical residues. The roman numbers refer to the predicted transmembrane α-helices of LmrA. The ABC signature sequence and Walker A/B motifs are indicated. (-) Gaps introduced to optimize the alignment.

Fig. 2. Expression of *lmrA* in the drug-sensitive *E.coli* strain CS1562. Total RNA from cells harboring pGK13 control vector (lane 1) and pGKLmrA (lane 2) was analyzed by Northern blotting using an *lmrA*-specific DNA probe. The arrow indicates the position of the *lmrA* mRNA. Transcript sizes (kilobase) were estimated using an RNA ladder.

Fig. 3. Ethidium transport in *E.coli* CS1562 with (pGKLmrA) and without (pGK13) expression of LmrA. Ethidium was added to washed cell suspensions at a final concentration of 50 μM. Cells were energized by the addition of 10 mM glucose (G).

Fig. 4. Daunomycin transport in inside-out membrane vesicles. (A) Uptake of daunomycin (3.8 μM, final concentration) in membrane vesicles prepared from *E.coli* CS1562/pGKLmrA (closed symbols) and *E.coli* CS1562/pGK13 (open symbols), in the presence of valinomycin plus nigericin (each at 1 nmol/mg of protein), 5 mM creatine-phosphate, and 1 mM ATPγS (○,●) or 1 mM ATP plus 0.1 mg/ml creatine kinase (□,■). (B) Effect of inhibitors on daunomycin uptake in inside-out membrane vesicles of LmrA-expressing cells. Inhibitors were included in the assay at a final concentration of 50 μM. The initial rate of ATP-dependent daunomycin uptake in membrane vesicles over the first 60 s was measured and corrected for the uptake of substrate in the presence of ATPγS. The control uptake (100%) was 7 pmol/min.mg of membrane protein.

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containing the apl and ImrA genes will be described elsewhere. Nucleotide and protein sequences were analyzed by PCGENE (release 6.8. Genofit). Genbank/EMBL Data Bank accession number is U 63741

18. Plasmid pGKLmrA was constructed by subcloning a 2.3 kb SphI-PvuII fragment containing lmrA, into the plasmid pGKI3. Transformants of E. coli CS1562 (tolC6::Tn10) were selected on Luria broth supplemented with 25 mM glucose, 16 µg/ml tetracycline, and 9 µg/ml chloramphenicol. For Northern blotting, total RNA (30 µg) was fractionated on a formaldehyde-agarose gel, transferred to Qiabrane membrane (Qiagen) and hybridized to the 2.3 kb SphI-PvuII DNA fragment.

19. Inside-out membrane vesicles were prepared as described [S. V. Ambudkar, G. W. Zlotnick, B. P. Rosen, *J. Biol. Chem.* 259, 6142 (1984)] and resuspended in 50 mM potassiurn-HEPES (pH 7.5) supplemented with 3 mM $MgSO_4$. Ethidium transport was measured using fluorescence spectrometry [L. L. Grinius and E. B. Goldberg, *J Biol. Chem.* 269, 2999 (1994)]. The uptake of [3H(G)]daunomycin (96.2 GBq/mmol, New England Nuclear) and APD-[21-3H]ajmalinium (46 GBq/rnmol) was determined in a filtration assay [S. R. Schlernmer and F. M. Sirotnak, J. *Biol. Chem.* 269, 31059 (1994)].

20. The initial rate of ATP dependent daunomycin uptake in membrane vesicles over the first 60 s was measured and corrected for the uptake of substrate in the presence of ATPγS. The control uptake rate (100%) was 7 pmolmin.mg of protein.

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Table 1. Effect of *ImrA* gene expression on the relative resistance to drugs of *E.coli* CS1562.

Drug	Relative Resistance
Ethidium	41 ± 6
Daunomycin	32 ± 5
Rhodamine 6G	45 ± 8
TPP^+	54 ± 5

Relative resistances were determined by dividing the IC_{50} (the drug concentration required to inhibit the growth rate by 50%) for cells harboring pGKLmrA, by the IC_{50} for control cells harboring pGK13. The latter values varied between 4 and 5 μM for the drugs tested.

CLAIMS

1. A recombinant or isolated nucleic acid molecule derived from a prokaryotic gene, said recombinant nucleic acid molecule encoding at least a specific and/or functional part and/or derivative of a transporter protein, which transporter protein has functional and/or structural similarity with the P-glycoprotein encoded by the human MDR1 gene.

2. A nucleic acid molecule according to claim 1, wherein the structural similarity with the human P-glycoprotein is at least 40% overall.

3. A nucleic acid molecule according to claim 1 or 2, wherein the structural similarity with the human P-glycoprotein comprises at least 30% identity overall.

4. A nucleic acid molecule according to any one of the claims 1-3, which encodes ATP-dependent efflux pump activity when expressed in a suitable host cell.

5. A nucleic acid molecule according to anyone of the claims 1-4, encoding a transmembrane protein.

6. A nucleic acid molecule according to anyone of the aforegoing claims, originating from a lactic acid bacterium.

7. A nucleic acid molecule according to claim 6, originating form the genus *Lactococcus*.

8. A nucleic acid molecule according to claim 7, originating from *Lactococcus lactis*.

9. A nucleic acid molecule according to anyone of the aforegoing claims, encoding at least a part of the protein according to figure 1.

10. A polypeptide obtainable by transcription and/or translation of a nucleic acid molecule according to anyone of the aforegoing claims.

11. A recombinant vector comprising a nucleic acid molecule according to any one of claims 1-9 and suitable regulatory elements for expression of the nucleic acid molecule and/or elements for transfer into host cells and/or replication in host cells.

12. A recombinant host cell comprising a nucleic acid molecule according to anyone of claims 1-9 and/or a vector according to claim 11.

13. A recombinant host cell comprising a transmembrane prokaryotic polypeptide having fuctional and/or structural similarity with the human P-glycoprotein encoded by the MDR-1 gene.

14. A method for producing a functional polypeptide having functional or structural similarity with the human P-glycoprotein encoded by the MDR1 gene, comprising culturing a recombinant host cell according to claim 12 or 13 in a suitable culture medium and harvesting the polypeptide from the culture.

15. A polypeptide having functional or structural similarity with the human P-glycoprotein encoded by the MDR1 gene obtainable by a method according to claim 14.

16. A recombinant or synthetic polypeptide comprising at least so many amino acid residues as to be functional as or specific for a polypeptide having functional or structural similarity with the human P-glycoprotein encoded by the MDR1 gene.

17. An antibody or a specific binding part and/or derivative thereof recognizing an epitope associated with a polypeptide according to any one of claims 10, 15 or 16.

18. Use of a polypeptide according to any one of claims 10, 15 or 16 or a cell according to any one of claims 12 or 13 in determining whether a substance can inhibit or escape multi drug resistance.

19. Method for determining whether a substance is capable of inhibiting transport of cytotoxic substances from a cell, comprising contacting said substance with a polypeptide according to any one of claims 10, 15 or 16 or contacting said substance with a cell according to any one of claims 12 or 13.

20. Method for providing cells with (additional) multidrug resistance, comprising providing the cells with a transmembrane polypeptide according to any one of claims 10, 15 or 16.

21. A method according to claim 20, whereby the transmembrane polypeptide is provided by expression in the cell of a nucleic acid according to any one of claims 1-9, or of a recombinant vector according to claim 11.

Fig. 1

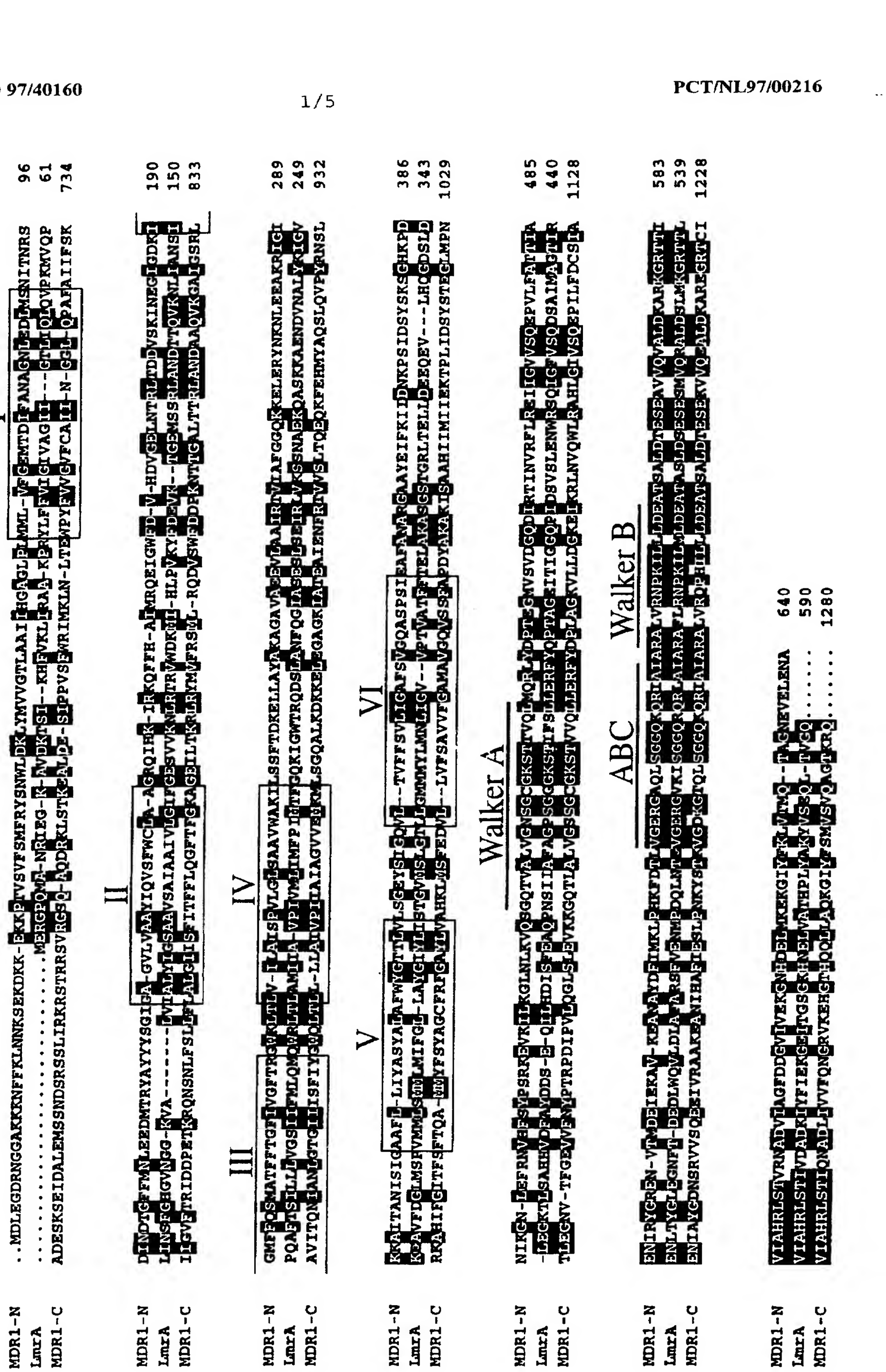
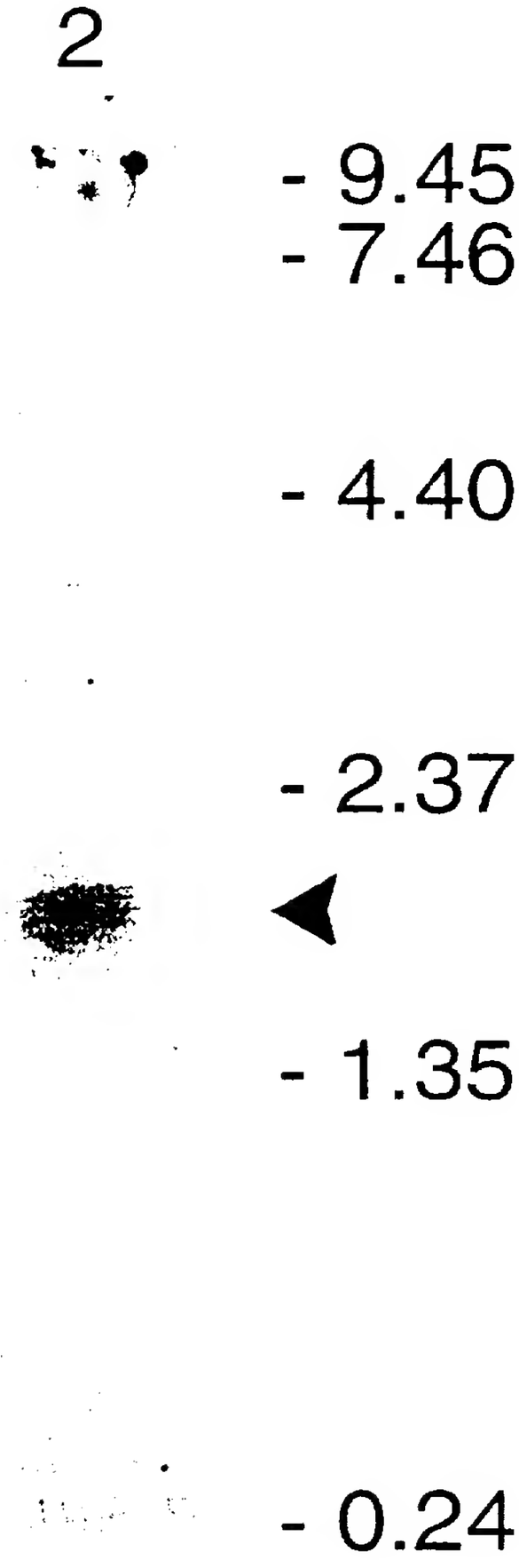
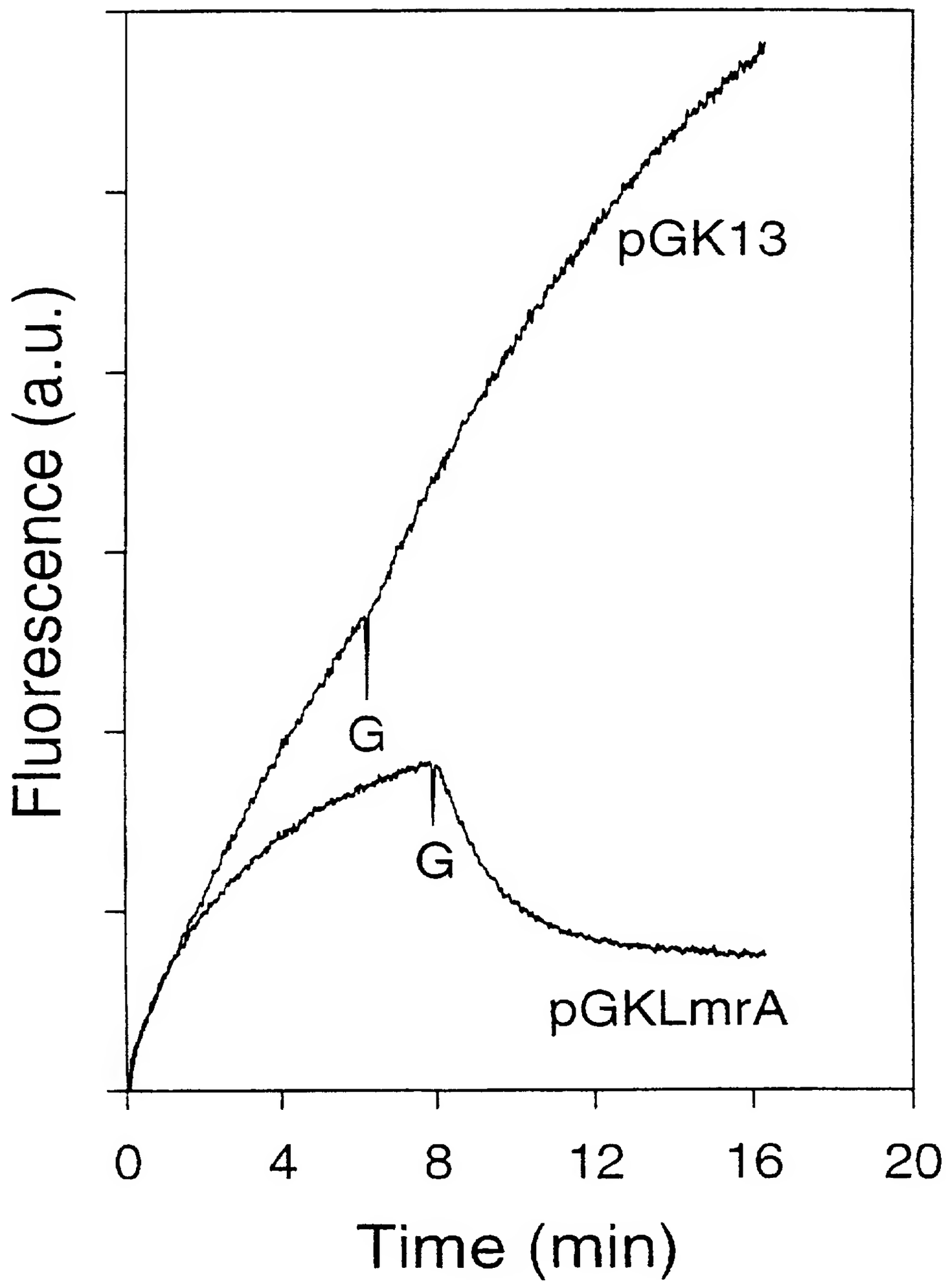
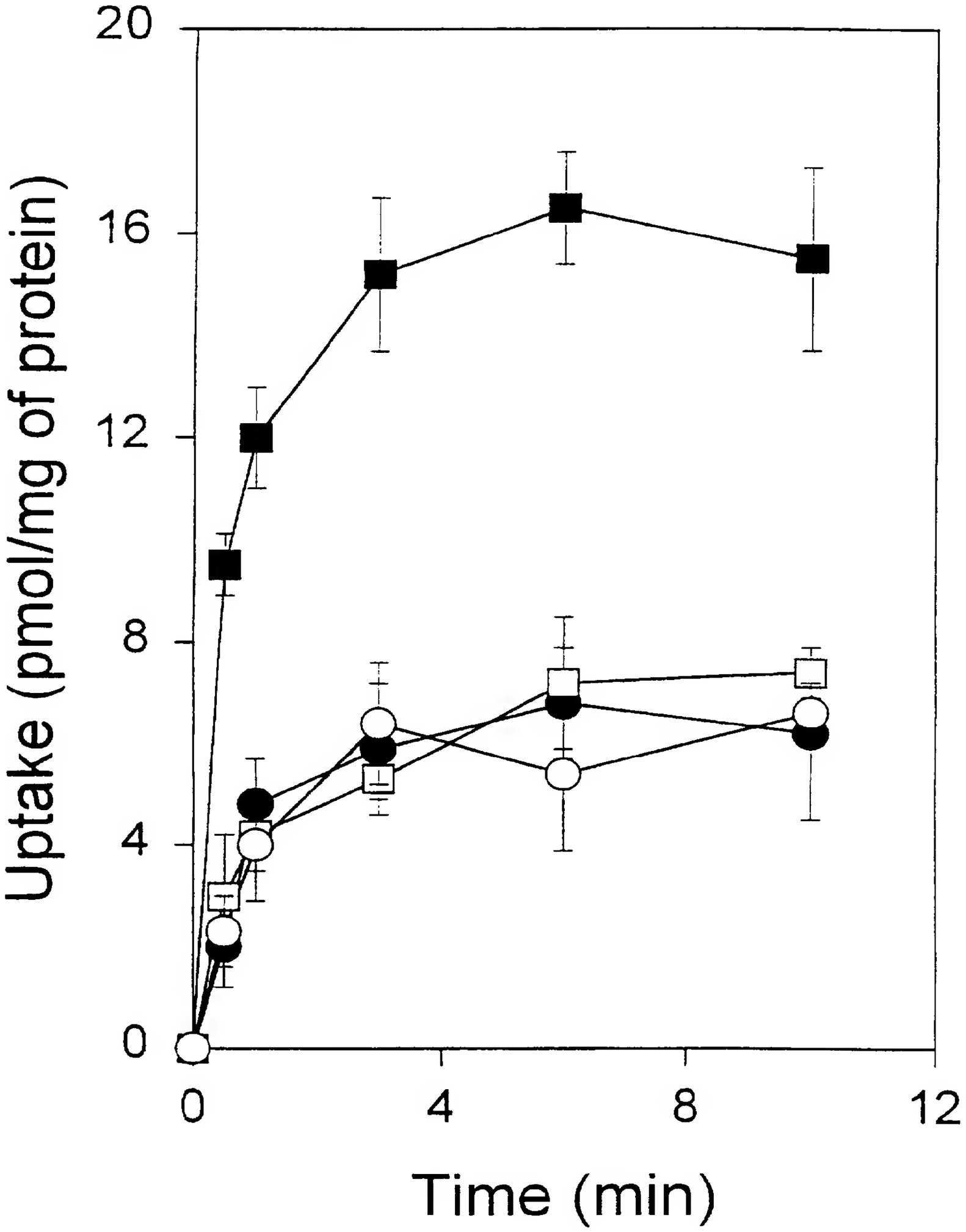
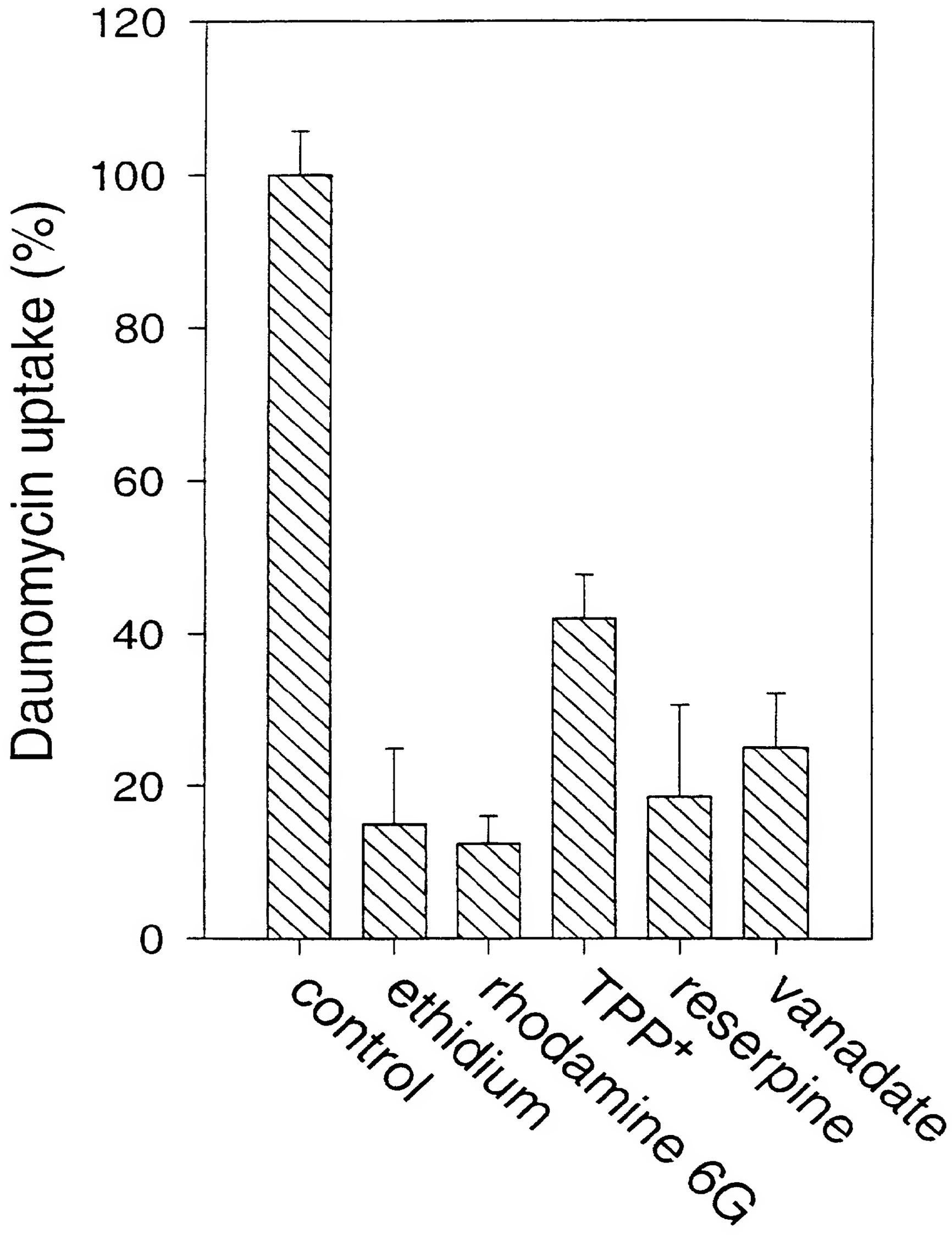


Fig. 2




Fluorescence (a.u.)
pGK13
G
G
pGKLmrA
0
4
8
12
16
20
Time (min)


20
16
12
8
4
0
Uptake (pmol/mg of protein)
0
4
8
12
Time (min)


120
100
80
60
40
20
0
Daunomycin uptake (%)
control
ethidium
rhodamine 6G
TPP+
reserpine
vanadate

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 97/00216

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/31 C12N15/70 C07K14/315 C12N1/21 C07K16/12
C12Q1/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-/--	

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
19 September 1997	07.10.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Montero Lopez, B

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International A ation No
PCT/NL 97/00216

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HENK BOLHUIS ET AL.: "The lactococcal lmrP gene encodes a proton motive force-dependent drug transporter" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 44, 3 November 1995, MD US, pages 26092-26098, XP002015107 cited in the application see abstract see page 26092, left-hand column, paragraph 1 - page 26093, left-hand column, paragraph 1 see page 26093, left-hand column, paragraph 3 see page 26093, right-hand column, last paragraph - page 26095, left-hand column, paragraph 1 see page 26095, left-hand column, paragraph 3 - page 26096, left-hand column, paragraph 1 see page 26096, right-hand column, paragraph 3 - page 26098, left-hand column, paragraph 1 ---	1,5-8, 10-21
X	ALAIN DEVAULT ET AL.: "Two members of the mouse mdr gene family confer multidrug resistance with overlapping but distinct drug specificities" MOLECULAR AND CELLULAR BIOLOGY, vol. 10, no. 4, April 1990, WASHINGTON US, pages 1652-1663, XP002015108 see abstract see page 1654, left-hand column, paragraph 3 - page 1661, right-hand column, paragraph 1 ---	1-5,9-21
A	HENK BOLHUIS ET AL.: "Proton motive force-driven and ATP-dependent drug extrusion systems in multidrug-resistant Lactococcus lactis" JOURNAL OF BACTERIOLOGY, vol. 176, no. 22, 1 November 1994, pages 6957-6964, XP000603563 cited in the application see abstract see page 6962, right-hand column, paragraph 2 see page 6963, left-hand column, paragraph 3 ---	1-21

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GONZALO CASTILLO ET AL.: "A homologue of the mammalian multidrug resistance gene (mdr) is functionally expressed in the intestine of Xenopus laevis" BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1262, no. 2-3, 1995, pages 113-123, XP000603630 see abstract; figure 2 see page 118, left-hand column, paragraph 2 - page 119, left-hand column, paragraph 1 see page 121, right-hand column, paragraph 1 - page 122, left-hand column, paragraph 2 ---	1-21
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P,X	BOLHUIS, HENK ET AL: "Multidrug resistance in Lactococcus lactis: evidence for ATP-dependent drug extrusion from the inner leaflet of the cytoplasmic membrane" EMBO J. (1996), 15(16), 4239-4245 CODEN: EMJODG;ISSN: 0261-4189, 1996, XP002036836 see abstract see page 4240, left-hand column, last paragraph - page 4241, left-hand column, paragraph 1 see page 4244, left-hand column, paragraph 3 - right-hand column, paragraph 2 ---	13-16, 18,19
P,X	W.N. KONINGS ET AL.: "The role of trnasport processes in survival of lactic acid bacteria" ANTONIE VAN LEEUWENHOEK, vol. 71, no. 1-2, February 1997, pages 117-128, XP002036837 see abstract see page 122, left-hand column, paragraph 2 - page 126, left-hand column, paragraph 3 -----	13-16, 18,19